JOURNAL OF AGRICULTURAL RESEARCH

VOL. 77 WASHINGTON, D. C., JULY 15, 1948 NO. 2

SESQUIDIPLOID F_1 HYBRIDS OF LYCOPERSICON ESCULENTUM AND L. PERUVIANUM [1]

By G. W. BOHN [2]

Plant pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Lycopersicon peruvianum (L.) Mill.,[3] a green-fruited wild tomato from South America, possesses several characters that would be valuable if they could be incorporated into tomato stocks possessing cultural size and quality. The determination of mosaic klendusity in a collection of *L. peruvianum* var. *dentatum* Dun. (*L. chilense* Dun.) and the successful hybridization of this collection with *L. esculentum* Mill. by Holmes (*8*, *9*) [4] stimulated interest in this species as a potential source of desirable economic characters. Virgin (*25*), Blood (*3*), and others reported collections of *L. peruvianum* to be resistant to curly top, and Wright and Lincoln (*28*) and others found some collections to be resistant to defoliation diseases. Bailey (*2*) and others reported nematode resistance in this species. Alexander, Lincoln, and Wright [5] surveyed this and other species in the genus *Lycopersicon* for reactions to several tomato diseases. Smith (*23*) and others reported that some collections of *L. peruvianum* are resistant to spotted wilt. Reynard and Kanapaux (*21*) and Lincoln and others (*12*) stated that this species contains considerably more ascorbic acid than does *L. esculentum*. Yeager and Purinton (*30*) recently reported high ascorbic acid content in lines derived from a cross between *L. esculentum* and *L. peruvianum*.

Observations on natural infestations among mixed species plantings at Cheyenne, Wyo., indicated that, like the commercial tomato, *Lycopersicon peruvianum* is attacked by tomato psyllids (*Paratrioza cockerelli* (Sulc)) but, unlike the commercial tomato, it does not develop marked symptoms of psyllid yellows.

F_1 hybrids were obtained fairly readily from crosses of *Lycopersicon esculentum* with certain collections of *L. peruvianum* by Holmes (*9*),

[1] Received for publication October 7, 1947.

[2] Thanks are extended to Paul Hepler, formerly junior scientific aid, Division of Fruit and Vegetable Crops and Diseases, for able assistance with all phases of the experimental work in 1944 and 1945; to Paul Desjardins and Robert Hildreth, formerly junior scientific aids, who made the pollinations in 1946; and to Mary E. Riner, formerly scientific aid, who recorded the data in 1946.

[3] The species name, unless stated otherwise, is used herein in its broad sense and includes the typical species and its varieties.

[4] Italic numbers in parentheses refer to Literature Cited, p. 52.

[5] ALEXANDER, L. J., LINCOLN, R. E., and WRIGHT, V. A SURVEY OF THE GENUS LYCOPERSICON FOR RESISTANCE TO THE IMPORTANT TOMATO DISEASES OCCURRING IN OHIO AND INDIANA. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 136, pp. [51]–85. 1942. [Processed.]

Wright and Lincoln (*28*), Yeager and Hepler (*29*), Virgin (*26*), and Lesley and Lesley (*11*). Certain other collections of *L. peruvianum* did not respond to the usual techniques of hybridization. Cross-pollinations between *L. esculentum* and this type of *L. peruvianum* made by the writer at the University of Missouri, Columbia, Mo., in 1935 failed to produce seeded fruits, as did those of Lesley (*10*), MacArthur and Chiasson (*15*), and several other workers at various locations. Hybridization of *L. esculentum* with collections that do not cross readily is desired because such collections have factors for resistance that do not occur in those that do cross readily with *L. esculentum*.

V. R. Boswell[6] suggested to the writer that hybrids between *Lycopersicon esculentum* and "difficult" collections of *L. peruvianum* might be obtained more readily at the Cheyenne Horticultural Field Station at Cheyenne, Wyo., than at other locations. The environmental conditions there are particularly favorable for fruit setting and seed production in the tomato (*18*). Since 1943, when these investigations were started, Smith (*22*) and McFarlane, Hartzler, and Frazier (*16*) have reported hybrids from crosses of "difficult" collections of *L. peruvianum* with *L. esculentum* by culturing young embryos from immature fruits on aseptic culture media. Porte and Walker (*20*) have obtained hybrids by using a small-fruited collection of *L. esculentum*, and Cooper and Brink (*5*) have obtained viable seeds from the cross tetraploid *L. pimpinellifolium* × diploid *L. peruvianum*. The results of the writer's experiments are reported herein.

MATERIALS AND METHODS

In preliminary experiments with diploids in 1943, two collections of *Lycopersicon peruvianum* (P. I.[7] 126944 and P. I. 128645[8]) that were known to cross with *L. esculentum* with difficulty or not at all were cross-pollinated reciprocally with several horticultural varieties of *L. esculentum*[9] and with *L. pimpinellifolium* (Jusl.) Mill. P. I. 79532. Many fruits and a few abortive seeds, but no hybrid seedlings, were obtained. These data are not reported in detail. After the failure to obtain hybrids from cross-pollinations between diploid races, experiments were conducted with diploid and tetraploid races.

The varieties Danmark (*1*) (early, determinate) and Waltham Forcing[10] (early, indeterminate) were used to represent *Lycopersicon esculentum* because they are prolific and early in pot cultures in the greenhouse at Cheyenne and would therefore yield more data per unit of space and per unit of time than other varieties. *L. peruvianum* P. I. 128645 was used in crosses with both of these horticultural varieties. Tetraploid seedlings of each of these three varieties were obtained from seeds soaked in colchicine solution as reported else where (*4*).

[6] Principal horticulturist, Division of Fruit and Vegetable Crops and Diseases.

[7] P. I. is used herein to indicate plant introductions imported through the Division of Plant Exploration and Introduction or its precursors.

[8] Received from H. L. Blood, formerly plant pathologist, Division of Fruit and Vegetable Crops and Diseases.

[9] Bay State, Bonny Best, Bounty, Break o'Day, Danmark, Earliana, Globe, Oxheart, Ponderosa, and Waltham Forcing.

[10] Obtained from Burnett–Seedsmen, Inc., New York, N. Y., 1943.

In 1943 and 1944 the plants were obtained directly from colchicine-treated or nontreated seeds. All plants from treated seeds that were used in the breeding work were identified as tetraploids by chromosome counts at meiosis in acetocarmine smears of pollen mother cells. Because the autotetraploids were readily identified by the gross morphological characters described by Winkler (*27*) and others, it was necessary to count the chromosomes only in plants actually used in the breeding work. Three plants of each variety from nontreated seeds were similarly identified as diploids. In 1943 only diploid races of *Lycopersicon esculentum* and diploid and tetraploid *L. peruvianum* were available. In the experiment conducted in the greenhouse during the winter of 1944, 30 tetraploid plants and 12 diploid plants of each variety of *L. esculentum* and of *L. peruvianum* were used.

For later experiments, conducted in the greenhouse in 1945 and 1946, seedlings from self-pollinated tetraploids and diploids of *Lycopersicon esculentum* and of *L. peruvianum* were used. Three or more plants of each line were verified as tetraploid or diploid by chromosome counts. In addition, a few plants of diploid and tetraploid *L. pimpinellifolium* P. I. 79532 and its diploid and amphidiploid (allotetraploid) [11] hybrid with *L. esculentum* variety Marglobe were used in crosses with *L. peruvianum*. The tetraploids and amphidiploids were obtained from colchicine-treated seeds. These and the diploids were identified by chromosome counts. *L. pimpinellifolium* and its hybrid with *L. esculentum* were used to determine whether they might serve as bridges in the attempt to transfer chromosome material from *L. peruvianum* to *L. esculentum*.

A few plants were grown in the field, but most of them were grown in 2-gallon, glazed-stone jars or 7-inch clay pots of soil in the greenhouse. The plants cultured in the greenhouse were maintained in a vigorous condition by adding 100 ml. of a nutrient solution containing 200 p. p. m. of N and 100 p. p. m. each of P and K made from commercial ammonium sulfate, treble superphosphate, and potassium sulfate at approximately 10-day intervals.

In preliminary experiments in both the field and greenhouse pollinations were made on emasculated flowers (except those selfed) that were protected from contamination by foreign pollen with paper or cloth bags. In later experiments performed entirely in the greenhouse such contamination was controlled by other methods. The plants were sorted into groups according to use; the pistillate parents were separated from the staminate ones by space on the greenhouse bench. Some plants were used alternately as staminate and pistillate parents by transferring them from one group to the other. With a few exceptions (see p. 44) all flowers on the pistillate parents were emasculated on the day before normal anthesis. The success of this method of control was demonstrated by the very rare occurrence of contamination under conditions in which such contamination could be readily determined.

[11] Although the tetraploid hybrid from *Lycopersicon esculentum* × *L. pimpinellifolium* produces progenies that segregate unit characters in ratios typical of autotetraploids, the present usage of species names is followed and the 48-chromosome hybrid is therefore considered to be an amphidiploid (allotetraploid) rather than a tetraploid (autotetraploid).

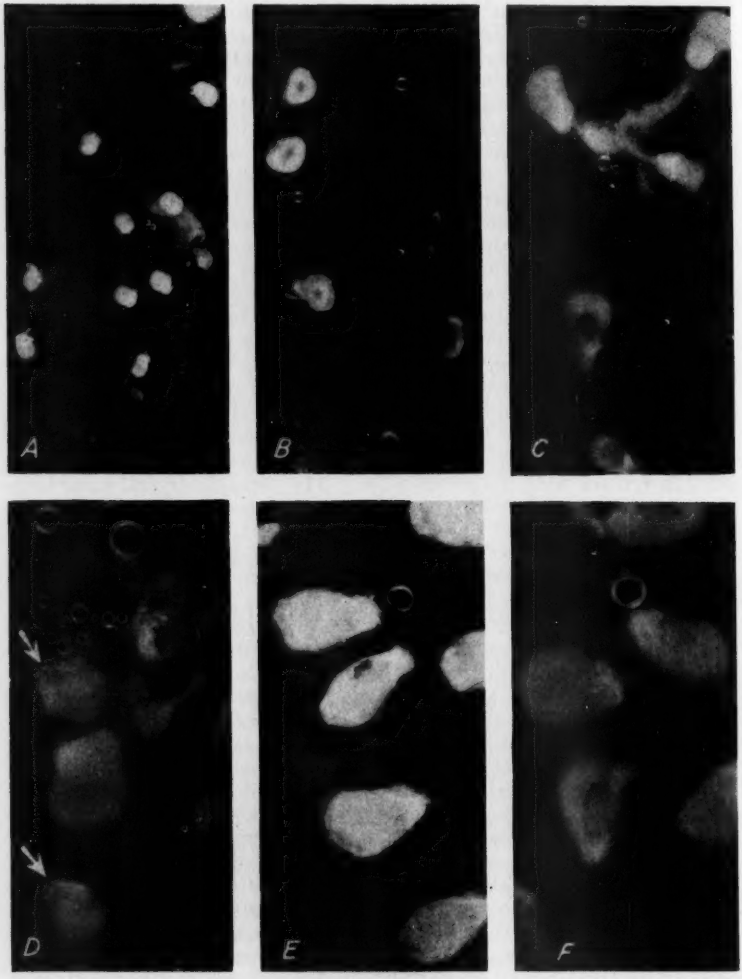

FIGURE 1.—Seed masses compressed in petri dishes to show the arbitrarily selected size classes of tomato ovules and seeds: *A*, Class 1, ovules barely or not at all enlarged; *B*, class 2, ovules slightly enlarged; *C*, class 3, ovules moderately enlarged; *D*, class 4, indicated by arrows, ovules much enlarged (poorly filled seeds); *E* and *F*, class 5, seeds large, plump. The larger seeds in *D* also belong to class 5. The actual crosses that were made to obtain the ovules and seeds shown were as follows: *A*, 2*n L. esculentum* ♀ × 4*n L. esculentum* ♂; *B*, 2*n L. esculentum* ♀ × 2*n L. peruvianum* ♂; *C*, 4*n L. esculentum* ♀ × 4*n L. peruvianum* ♂; *D*, 4*n L. esculentum* ♀ × 2*n L. peruvianum* ♂; *E*, 2*n L. esculentum* selfed; *F*, 4*n L. esculentum* selfed. × 5.4.

Pollen was collected on alcohol-cleaned, dry slides with the use of an electric vibrator and placed on the stigmas on the day anthesis would normally occur and again 2 days later. Some flowers were pollinated the day before and the day after anthesis. Usually two or three flowers but occasionally more were used on a single cluster; the unused flowers were removed before anthesis. Flowers on the staminate parents were self-pollinated at the time pollen was collected for the cross-pollinations.

To determine the relative sizes of ovules or seeds, the seed mass in each fruit was removed to the lid of a 90-mm. petri dish and the inverted dish was pressed against the seed mass to render the individual ovules or seeds readily visible. In most cases the ovules and seeds were grouped into the five arbitrary size classes shown in figure 1. The size classes shown were used for rating seed masses from fruits of diploid *Lycopersicon esculentum*. For rating seed masses from other pistillate parents, large seeds in fruits from self-pollinated flowers of the respective pistillate parents were considered to be size 5. The extra large, plump seeds from tetraploid *L. esculentum* selfed (fig. 1, *F*) were considered to be size class 5 for this pistillate parent.

RESULTS

Because similar results were obtained from all trials of any one type of cross-pollination that was repeated, the data from all experiments have been summarized (table 1) and are discussed together. The data in table 1 are grouped according to the chromosome complements of the plants crossed to agree with the sequence in the discussion. Although minor differences occurred, Danmark, Waltham Forcing, and other commercial varieties yielded similar results in all combinations; the data from them are therefore combined under *Lycopersicon esculentum*. This procedure necessitated the use of weighted averages for the fruit weights and average numbers of seeds in fruits. The weighted averages were obtained by using equal numbers of fruits of Danmark and Waltham Forcing as composite samples. Although size 5 seeds frequently produced larger percentages of seedlings than did size 4 seeds, the data on size 4 and size 5 seeds, including the average numbers in a fruit and the percentages that produced seedlings, were combined for brevity.

SELF-POLLINATED DIPLOIDS

Self-pollinated flowers of diploid races of *Lycopersicon esculentum*, *L. pimpinellifolium*, and their F_1 hybrid set fruits with normal complements of well-developed seeds during both the summer and the winter (fig. 1, *E*, and table 1). The seed number in the hybrid was intermediate between the seed numbers in the parent species. Self-pollinated and naturally pollinated flowers of diploid *L. peruvianum* set fruits with normal complements of well-developed seeds during the summer, but they failed to set fruits or set fruits with few seeds during the winter (table 1). In addition to the results reported in table 1, 110 self-pollinated flowers on 6 plants produced 7 small, single-seeded fruits during the winter of 1943 and naturally pollinated flowers set no fruit. These results were obtained whether the flowers were pollinated first at anthesis or 1 or 2 days before anthesis.

TABLE 1.—*Results from self- and cross-pollinations with diploid and tetraploid Lycopersicon esculentum, L. pimpinellifolium, L. esculentum × L. pimpinellifolium F_1, and L. peruvianum at Cheyenne, Wyo.*

Type of cross and species of pistillate parent	Species of staminate parent	Season	Flowers pollinated	Flowers setting fruits	Average weight of fruits	Fruits with largest ovules or seeds as indicated					Average seeds per fruit, sizes 4 and 5	Seeds planted, sizes 4 and 5	Seeds producing seedlings
						Size 1	Size 2	Size 3	Size 4	Size 5			
			Number	*Percent*	*Grams*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Self-pollinated diploids:													
L. esculentum	*L. esculentum*	Winter	50	90	61.3	0	0	0	0	45	122	320	78
		Summer	49	78		0	0	0	0	38	176	121	76
L. pimpinellifolium	*L. pimpinellifolium*	Winter											
		Summer	48	92	1.1	0	0	0	0	44	31		
L. esculentum×*L. pimpinellifolium* F_1.	*L. esculentum*×*L. pimpinellifolium* F_1.	Winter	25	96		0	0	0	0	24			
		Summer	43	91	7.0	0	0	0	0	39	61		
L. peruvianum	*L. peruvianum*	Winter	100	5	2.3	0	0	0	1	4	47		
		Summer								46	78	72	81
Self-pollinated tetraploids:													
L. esculentum	*L. esculentum*	Winter	162	53	54.0	0	0	0	0	85	43	955	39
		Summer	44	82		0	0	0	0	36	40	126	47
L. pimpinellifolium	*L. pimpinellifolium*	Winter											
		Summer	17	77	1.1	0	0	0	0	13	9		
L. esculentum×*L. pimpinellifolium* F_1.	*L. esculentum*×*L. pimpinellifolium* F_1.	Winter											
		Summer	40	93	8.4	0	0	0	0	37	31		
L. peruvianum	*L. peruvianum*	Winter	48	75	4.5	0	0	0	0	36	34	280	69
		Summer								10	24	36	56
Diploids×diploids (interspecific):													
L. esculentum	do	Winter	20	100	53.1	1	10	7	0	2	.1	2	0
		Summer	66	92		15	33	11	0	2	.03	2	0
L. pimpinellifolium	do	Winter											
		Summer	48	83	1.0	1	39	0	0	0	0		
L. esculentum×*L. pimpinellifolium* F_1.	do	Winter	7	100		0	7	0	0	0	0		
		Summer	35	91	4.8	0	30	0	1	1	.06	2	0
L. peruvianum	*L. esculentum*	Winter	14	0									
		Summer	33	0									
Tetraploids×tetraploids (interspecific):													
L. esculentum	*L. peruvianum*	Winter	127	80	48.1	0	18	81	1	1	.06	6	0
		Summer	9	100		0	0	9	0	0	0		
L. pimpinellifolium	do	Winter											
		Summer	12	92	1.0	0	3	8	0	0	0		
L. esculentum×*L. pimpinellifolium* F_1.	do	Winter											
		Summer	16	81	5.0	0	3	10	0	0	0		
L. peruvianum	*L. esculentum*	Winter	88	3	1.0	3	0	0	0	0	0		
		Summer											

Diploids×tetraploids (interspecific):													
L. esculentum	*L. peruvianum*	Winter	177	91	38.6	.13	82	62	2	2	.06	10	
		Summer	87	92		0	10	70	0	0	0		
L. pimpinellifolium	do	Winter											
		Summer	21	76		5	10	1	0	0	0		
L. esculentum×*L. pimpinellifolium* F_1	do	Winter	29	97		0	27	1	0	0	0		
		Summer	8	100	5.0	0	5	3	0	0	0		
L. peruvianum	*L. esculentum*	Winter	42	0									
		Summer	148	5		8	0	0	0	0	0		
Tetraploids×diploids (interspecific):													
L. esculentum	*L. peruvianum*	Winter	20	85	70.4	0	0	0	2	15	19	228	8
		Summer	124	88		0	0	30	11	68	9	186	24
L. pimpinellifolium	do	Winter											
		Summer	19	84	1.8	0	0	0	1	15	8		
L. esculentum×*L. pimpinellifolium* F_1	do	Winter											
		Summer	31	65	7.4	0	0	0	0	20	23		
L. peruvianum	*L. esculentum*	Winter	52	0									
		Summer	55	7		0	0	3	1	0	12	47	0

The unfruitfulness of diploid *Lycopersicon peruvianum* during the winter apparently resulted from the effect of environmental factors on the young pistil or on the flower after pollination. Detrimental effects of high temperature comparable with those reported by Lesley and Lesley (*11*) would not seem to be the limiting factor in these experiments. The plants were vigorous and flowered profusely. The flowers appeared to be normal at anthesis and produced abundant pollen that functioned in crosses with *L. esculentum*. Furthermore, the day temperature in the greenhouse during the winter usually was kept within the range 75° to 85° F. and exceeded 90° for only brief periods on sporadic occasions. Day temperature in the greenhouse during the summer, when this species sets fruits, was usually somewhat higher and exceeded 90° frequently. No irregularities were observed in meiosis in the pollen mother cells of plants on which chromosome counts were made. The failure of diploids of this species to set fruits during the winter and their ability to set abundant seeded fruits in both the greenhouse and the field at Cheyenne during the summer suggest that light may be an important factor.

The percentages of seedlings produced by seeds of the diploid races planted in steamed soil served as checks for evaluating the percentages of seedlings produced by other seeds.

SELF-POLLINATED TETRAPLOIDS

Relatively fewer self-pollinated flowers of tetraploid *Lycopersicon esculentum* set fruits than did those of the diploid in the winter, but they set fruits as readily in the summer (table 1). The fruits of the self-pollinated tetraploids were smaller and contained fewer but larger seeds (fig. 1, *F*) than the fruits of the corresponding diploids. Relatively fewer of the seeds of the tetraploids planted in steamed soil produced seedlings than did those of the corresponding diploids. The tetraploid seedlings grew more slowly and were readily distinguished by their thicker stems and broader cotyledons.

These results are in agreement with those obtained from autotetraploids of other varieties of *Lycopersicon esculentum* by other investigators. Upcott (*24*) attributed the low fertility of autotetraploid *L. esculentum* to the formation of quadrivalents and numerical nondisjunction at meiosis. This was supported experimentally by Oka's (*19*) report that 8 percent of the seeds from self-pollinated tetraploids yielded heteroploid seedlings with 49 to 54 chromosomes. Lindstrom and Koos (*14*) attributed extreme sterility in a tetraploid derived from a haploid to complete homozygosity. It is of interest to note the relatively numerous seeds in fruits of tetraploid plants of the Danmark and Waltham Forcing varieties used in the present experiments. Danmark yielded an average of 41.3 seeds in size classes 4 and 5 in fruits from tetraploid plants derived from colchicine-treated seeds and an average of 30.0 seeds in fruits from plants derived from the S_1 (first selfed) generation. These tetraploids were obtained from several diploid plants in a line that had been inbred for 10 generations after it was received at the Cheyenne station. Tetraploid plants of Waltham Forcing were obtained from diploid plants in a line inbred for 2 generations after it was received. Fruits from these plants obtained from colchicine-treated seeds yielded an average of 44.5 seeds; those

from the S_1 generation yielded 42.7 seeds. Probably the degree of homozygosity in these inbred lines does not approach that in the tetraploid derived from the haploid.

The tetraploids of *Lycopersicon pimpinellifolium* yielded results similar to those obtained from *L. esculentum* except that the weight of the tetraploid fruits was as great as that of the diploid *L. pimpinellifolium* or greater (table 1). There were fewer seeds in a fruit, of course, than in a fruit of *L. esculentum*.

The amphidiploid hybrid between *Lycopersicon esculentum* and *L. pimpinellifolium* yielded results similar to those obtained from the parent species except that its fruit weight was definitely greater than that of the diploid hybrid (table 1). Although the proportion of self-pollinated flowers that set fruits was as high in the amphidiploid as in the diploid, the average number of seeds in a fruit was considerably lower. High fertility of the amphidiploid from this species cross has been reported by Lindstrom and Humphrey (*13*).

In contrast with flowers of diploid *Lycopersicon peruvianum*, the self-pollinated and naturally pollinated flowers of tetraploid *L. peruvianum* set seeded fruits readily in the greenhouse during the winter. Individual plants in 7-inch clay pots produced as many as 100 fruits. The tetraploids also set fruits in the field and greenhouse during the summer. The fruits were approximately twice as heavy as seedy fruits of the diploid. These results contrast with those obtained on fruit size in diploid and tetraploid *L. esculentum* and *L. pimpinellifolium* in these and other investigations.

The seeds were fewer and larger in fruits of tetraploid *Lycopersicon peruvianum* than in fruits of the diploid. As in the diploid, the number of seeds varied in different plants. Some of the tetraploids in the S_1 generation had only 1 or 2 seeds in a fruit. These may have been similar to the unfruitful autotetraploids of *L. esculentum* reported by Lindstrom and Humphrey (*13*); or they may have been heteroploids similar to those obtained by Oka (*19*). Chromosome counts were not made on the S_1 plants with exceptionally few seeds. Observations on meiosis in 3 of their more fertile sibs and in 30 tetraploids derived directly from colchicine-treated seeds showed that 1 pair of chromosomes frequently fails to synapse (fig. 2) or lags on the spindle at anaphases I and II in pollen mother cells. Seedling emergence of the self-pollinated tetraploids was less and somewhat slower than that of the self-pollinated diploids. However, the tetraploid seedlings were larger than the diploid ones and had thicker stems and broader cotyledons. The growth rate of tetraploid *L. peruvianum* seedlings approached that of its diploid, and its initial size advantage was maintained during early growth.

The results from the self-pollinations serve as a basis for evaluating those from the cross-pollinations.

DIPLOIDS × TETRAPLOIDS (INTRASPECIFIC)

A few intraspecific cross-pollinations were made between diploid and tetraploid races for comparison with the interspecific crosses. Five fruits set by 6 flowers of diploid *Lycopersicon esculentum* pollinated with pollen from its tetraploid contained only abortive ovules

795720—48——2

in size classes 1, 2, or 3 (fig. 1, *A*, *B*, and *C*). Twelve fruits set by 14 flowers of tetraploid *L. esculentum* treated with pollen from its diploid contained abortive ovules and 6 seeds. The seeds were similar in appearance to those obtained from self-pollinated tetraploid *L. esculentum*. They produced triploid seedlings. These results are in agreement with those reported by Cooper and Brink (*5*).

Although self-pollinated flowers of diploid *Lycopersicon peruvianum* usually failed to set fruits during the winter, 1 single-seeded fruit was obtained from 10 flowers of this race treated with pollen from tetraploid *L. peruvianum*. This seed produced a triploid plant that was intermediate between the diploid and tetraploid plants in appearance.

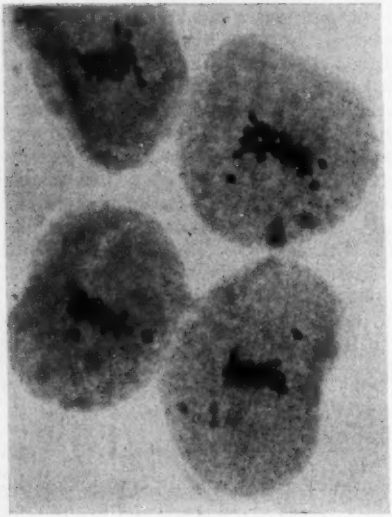

FIGURE 2.—Early anaphase I in microsporocytes of tetraploid *Lycopersicon peruvianum* showing unpaired chromosomes that failed to reach the plate area and were left in the cytoplasm. × 696.

DIPLOIDS × DIPLOIDS (INTERSPECIFIC)

As in earlier experiments, emasculated flowers of diploid plants of all of the varieties of *Lycopersicon esculentum* set fruits readily if pollinated with pollen from diploid *L. peruvianum* (table 1). Similar results were obtained if pollen from diploid *L. peruvianum* was applied to emasculated flowers of diploid *L. pimpinellifolium* or of its F_1 hybrid with *L. esculentum*. Most of the fruits contained ovules that were slightly or moderately enlarged (fig. 1, *B* and *C*). These failed to yield viable seedlings in steamed soil in the greenhouse. Selected size 3 ovules removed from ripe fruits and from young fruits 20 days after pollination and planted under aseptic conditions on nutrient-salt-dextrose agar also failed to show any indications of growth. The histological studies of Cooper and Brink (*5*) indicated that such ovules do contain small embryos. Furthermore, Smith (*23*) secured hybrid

seedlings from excised embryos that were removed from young fruits and cultured aseptically, and McFarlane, Hartzler, and Frazier (*16*) optained seedlings from similar ovules cleaned of ovarian residue but left intact. Possibly growth of the embryos was inhibited by the surrounding ovarian tissue in the studies reported herein, although an effort was made to remove fruit residue from the ovules by rubbing them on a sterile wood surface.

Four seeds classified in size classes 4 and 5 were obtained from 81 fruits from cross-pollinations between diploid *Lycopersicon esculentum* and diploid *L. peruvianum* (table 1). One of these seeds was similar in appearance to those from the self-pollinated diploid *L. esculentum*. As it yielded a seedling like the pistillate parent, it may have resulted from chance contamination by a single pollen grain of *L. esculentum* or from development of an unfertilized diploid megagamete. The other 3 seeds were extra large, green, and apparently immature. Two seeds of this type also occurred in single-seeded fruits from the cross between the diploid *L. esculentum* × *L. pimpinellifolium* F_1 hybrid and diploid *L. peruvianum*. The similarity of these seeds to those from the successful crosses between tetraploid *L. esculentum* and diploid *L. peruvianum* (p. 45) suggests that they may have resulted from fertilization of diploid megagametes. Like the latter, these seeds contained well-developed endosperms and small but differentiated embryos. The embryos were too weak to break through the seed coats of the intact seeds on moist blotters in a seed germinator. They enlarged after they were dissected from the seed coats and placed on clean blotters, but they failed to produce mature plants.

As in earlier experiments, flowers of diploid *Lycopersicon peruvianum* emasculated and treated with pollen from diploid *L. esculentum* failed to set fruits (table 1). The failure to obtain fruits from this cross during the winter probably is not significant because self-pollinated flowers of diploid *L. peruvianum* rarely set fruits during this season. However, flowers cross-pollinated during the summer, when self-pollinations were successful, dropped a few days after anthesis, as did emasculated, nonpollinated control flowers. During the winter of 1943 emasculated flowers of diploid *L. peruvianum* were pollinated with pollen from diploid *L. esculentum* and induced to set by applying to the pedicels 0.1-percent indolebutyric acid in lanolin emulsion. In 33 fruits (not included in table 1) obtained by this technique most of the ovules had enlarged very little or not at all. Two plump seeds produced in these fruits yielded seedlings identical with the pistillate parent. These results indicate that cross-fertilization did not occur. Whether this resulted from failure of the haploid pollen tubes of *L. esculentum* to grow normally in the diploid pistils of *L. peruvianum* was not determined.

TETRAPLOIDS × TETRAPLOIDS (INTERSPECIFIC)

Tetraploid *Lycopersicon esculentum*, tetraploid *L. pimpinellifolium*, and their amphidiploid hybrid pollinated with pollen from tetraploid *L. peruvianum* yielded results similar to those obtained from the same type of pollination with diploids (table 1 and fig. 1, *B* and *C*). Most of the fruits contained size 3 ovules. Six shriveled seeds in size class 4 obtained from 121 fruits from these crosses failed to yield

viable seedlings. They were not green like the seeds from the cross between diploids, but they were not dissected for comparison of internal tissues. Possibly the amphidiploid (allotetraploid) hybrid between *L. esculentum* and *L. peruvianum* could be obtained from this type of cross by Smith's (*23*) embryo-culture technique.

Emasculated flowers of tetraploid *Lycopersicon peruvianum* pollinated with pollen from tetraploid *L. esculentum* usually failed to set fruits (table 1). The few fruits that did set were much smaller than control fruits from self-pollinated flowers and contained nonenlarged ovules. Twelve fruits were set by 22 emasculated, cross-pollinated flowers late in the pollinating period when lower clusters of nonemasculated flowers on the same plants were cross-pollinated. These flowers and fruits were not included in table 1 because it seems likely that small amounts of pollen from the nonemasculated flowers reached the stigmas of some or all of them. Of the 12 fruits, 5 contained size 2 and size 3 ovules and 7 contained small numbers of plump seeds. Only tetraploid *L. peruvianum* seedlings were obtained from these plump seeds, indicating that some or all of the 12 fruits were set as the result of chance self-pollination. Although some of the fruits were seedless, it is not certain that they were set as the result of the application of pollen from tetraploid *L. esculentum;* they may have resulted from stimulation by pollen tubes from pollen of tetraploid *L. peruvianum* that reached the stigmas too late to permit fertilization and seed development to occur.

Numerous technical difficulties were encountered in the manipulation of both diploid and tetraploid *Lycopersicon peruvianum* as pistillate parents in crosses. The styles on these plants were long, slender, and curved, and the anthers were not easily separated from the abundant hairs on the bases of the styles. During emasculation the styles frequently broke off at the point of attachment to the ovary. After apparently successful emasculation of flowers the day before anthesis would occur, the long slender styles frequently dried out before the petals reflexed; these were not pollinated. A small proportion of the cross-pollinated flowers of tetraploid *L. peruvianum* recorded in table 1 may have dropped as a result of the drying of the styles after pollination, but most of them retained healthy styles for several days. There was little indication that stimulation followed applications of pollen from tetraploid *L. esculentum*. The flowers usually dropped 3 to 7 days after anthesis. Three very small fruits with nonenlarged ovules were obtained.

Because of the difficulties mentioned, pollen from tetraploid *Lycopersicon esculentum* was used on nonemasculated flowers of tetraploid *L. peruvianum*. The flowers were pollinated as soon as the styles extruded from the staminal cone, and pollinations were repeated daily until anthesis. An effort was made not to disturb flowers that had begun to shed pollen. It was considered possible that ovary stimulation and self-fertilization in a few ovules after self-pollination with a few pollen grains might permit fertilization in some ovules by microgametes from the applied *L. esculentum* pollen and the development of these ovules containing hybrid embryos. Flowers so treated usually dropped a few days after anthesis or remained attached to the plants without ovary enlargement. A few produced fruits containing various

numbers of plump seeds and ovules of different sizes. The latter contained complements of ovules and seeds similar to those in fruits from naturally pollinated flowers. Seeds from fruits that contained 17 (half the number obtained in fruits from self-pollinated flowers) seeds or fewer were planted in steamed soil in the greenhouse. None of the resulting seedlings was a hybrid; all were fertile *L. peruvianum* tetraploids comparable with those obtained after self-pollination.

The results from emasculated and nonemasculated flowers indicated that tetraploid *Lycopersicon esculentum* was impotent as a fruit-setting agent on tetraploid *L. peruvianum*. Whether this resulted from failure of fertilization to occur, from failure of the diploid pollen tubes of *L. esculentum* to grow in the tetraploid styles of *L. peruvianum*, or from some other cause was not determined. This type of cross-pollination showed little promise as a means of obtaining species hybrids.

DIPLOIDS × TETRAPLOIDS (INTERSPECIFIC)

Emasculated flowers of diploid *Lycopersicon esculentum*, diploid *L. pimpinellifolium*, and their diploid F_1 hybrid treated with pollen from tetraploid *L. peruvianum* set fruits readily. Most of the fruits contained ovules that were slightly to moderately enlarged (table 1 and fig. 1, *B* and *C*); the ovules were similar in appearance to those in fruits from cross-pollinations between lines with equal chromosome numbers. Four fruits from the *L. esculentum* parent contained three seeds in size class 4 and seven seeds in size class 5. These were similar in appearance to seeds in these size classes from self-pollinated *L. esculentum*, but they failed to produce seedlings in steamed soil.

Most of the flowers of diploid *Lycopersicon peruvianum* pollinated with pollen from tetraploid *L. esculentum* failed to set fruits. The results obtained during the winter probably are not significant, because self-pollinated flowers of diploid *L. peruvianum* rarely set fruits during this season. However, similar results were obtained during the summer when self-pollinated flowers set readily. Eight small fruits from this cross-pollination contained nonenlarged ovules (table 1). This cross-pollination showed little promise as a means of obtaining hybrids.

TETRAPLOIDS × DIPLOIDS (INTERSPECIFIC)

Flowers of tetraploid *Lycopersicon esculentum* pollinated by diploid *L. peruvianum* set fruits as readily as self-pollinated flowers (table 1). The average weight of these fruits was greater than that of fruits set on either diploid or tetraploid *L. esculentum* from applications of pollen from any other source (table 1). Tetraploid *L. pimpinellifolium* if used as the pistillate parent yielded similar results. A lower percentage of the flowers of the amphidiploid hybrid *L. esculentum* × *L. pimpinellifolium* F_1 set fruits, but the weight data were similar.

In contrast with fruits from other cross-pollinations, the fruits from these crosses contained some large, plump seeds (table 1 and fig. 1, *D*). These seeds differed in shape from those following self-pollinations (compare fig. 1, *D* and *F*). They had a distinctive green color that contrasted sharply with the light brown of seeds from self-pollinated diploid and tetraploid *Lycopersicon esculentum* and *L. pimpinellifolium* and with the reddish brown of seeds from self-pollinated

diploid and tetraploid *L. peruvianum*. With a few exceptions previously noted (p. 43), the enlarged ovules or seeds in fruits from other cross-pollinations did not possess this green color.

The large, green seeds from these successful crosses appeared to be immature. It was noted that the period from anthesis to fruit and seed maturity was considerably longer in diploid and tetraploid *Lycopersicon peruvianum* (70 to 80 days) than in diploid and tetraploid *L. esculentum* (50 to 60 days) under the conditions of these experiments. Fruits from cross-pollinated flowers picked ripe and stored in brown paper bags in the laboratory at 65° to 70° F. for 30 days yielded brown seeds that were apparently more nearly mature.

Comparatively few seeds classified as sizes 4 and 5 from the successful cross tetraploid *Lycopersicon esculentum* × diploid *L. peruvianum* produced seedlings (table 1). Higher percentages of seedlings were produced from seeds left in the fruits for 90 days after pollination than from those removed as soon as the fruits were ripe. On dissection, seeds that failed to germinate were found to have well-developed endosperms and comparatively small embryos. These embryos were apparently slow in initiating growth and too weak to break the seed coats.

The hybrid plants from this successful cross were vigorous and had morphological characters intermediate between those of the parents (fig. 3). In appearance they were very similar to those described by Porte and Walker (*20*). Counts of chromosomes at meiosis in 8 plants showed that these hybrids had 36 chromosomes. These counts and those of chromosomes in the parents (48 chromosomes in tetraploid *Lycopersicon esculentum* and 24 chromosomes in diploid *L. peruvianum*) showed that the hybrids were sesquidiploids (*7, p. 275*) (allotriploids) that contained 2 sets of chromosomes (24) from *L. esculentum* and 1 set of chromosomes (12) from *L. peruvianum*.

Vigorous hybrids were obtained also from the cross amphidiploid *Lycopersicon esculentum* × *L. pimpinellifolium* F_1 × diploid *L. peruvianum*. These plants could not be distinguished readily from plants from the cross tetraploid *L. esculentum* × diploid *L. peruvianum*. Although these plants might be expected to vary in appearance because of variations in the relative numbers of *L. esculentum* and *L. pimpinellifolium* chromosomes they contained, such variation was not observed among 10 plants grown in pots in the greenhouse.

No seeds from the cross tetraploid *Lycopersicon pimpinellifolium* × diploid *L. peruvianum* were planted.

Most of the flowers of tetraploid *Lycopersicon peruvianum* pollinated by diploid *L. esculentum* dropped a few days after pollination (table 1). Apparently, diploid *L. esculentum* functions poorly as a fruit-setting agent on tetraploid *L. peruvianum*. One fruit from this cross contained 47 size 4 seeds. These seeds failed to produce seedlings from plantings made in steamed soil. Similar results had been obtained in earlier experiments. These results suggest that this cross may yield hybrids from repeated trials or by the use of other parental varieties or techniques. However, seeds from fruits set by nonemasculated, cross-pollinated flowers produced only tetraploid *L. peruvianum* seedlings.

FIGURE 3.—Tomato plants grown in the greenhouse, Cheyenne, Wyo., summer, 1945: *A*, Tetraploid *Lycopersicon esculentum*; *B*, sesquidiploid; *C*, diploid *L. peruvianum*.

VARIATION IN LINES OF DIPLOID LYCOPERSICON PERUVIANUM P. I. 128645

Differences were obtained in the average number of seeds in fruits from crosses between tetraploid *Lycopersicon esculentum* and diploid *L. peruvianum* P. I. 128645 in the winter and in the summer (table 1). In the summer similar differences occurred in crosses involving different inbred lines from the same introduction of *L. peruvianum*. These data are presented in greater detail in table 2. The 12 S_1

TABLE 2.—*Results from pollinations of tetraploid Lycopersicon esculentum (varieties Danmark and Waltham Forcing), tetraploid L. pimpinellifolium, and tetraploid L. esculentum × L. pimpinellifolium F_1 with pollen from different inbred lines of diploid L. peruvianum P. I. 128645 in the greenhouse at Cheyenne, Wyo.*

Pistillate parent	Line No. of staminate parent	Flowers pollinated	Flowers setting fruits	Fruits with largest ovules or seeds as indicated					Average seeds per fruit, sizes 4 and 5	Seedlings planted	Seedlings producing seedlings
				Size 1	Size 2	Size 3	Size 4	Size 5			
L. esculentum:		*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Danmark	5	22	91	0	0	10	3	7	1.7	13	1
	6	20	90	0	0	2	1	15	7.9	61	29
	7	18	78	0	0	9	2	3	1.2	16	1
Waltham Forcing	5	11	82	0	0	1	2	6	5.6	19	2
	6	26	96	0	0	0	1	24	26.8	35	11
	7	27	85	0	0	8	2	13	3.8	42	1
L. pimpinellifolium	5	7	86	0	0	0	0	6	9.2	------	------
	6	6	83	0	0	0	0	5	11.6	------	------
	7	6	83	0	0	0	1	4	3.8	------	------
L. esculentum × L. pimpinellifolium F_1	5	14	50	0	0	0	0	7	23.3	------	------
	6	10	70	0	0	0	0	7	21.7	------	------
	7	7	83	0	0	0	0	6	20.0	------	------

plants of diploid *L. peruvianum* used during the winter and the 9 S_2 plants used during the summer all had their origin in a single plant of *L. peruvianum* P. I. 128645 self-pollinated in the greenhouse in 1943. The 9 S_2 plants used in the summer consisted of 3-plant progenies from 3 field-grown sibs of the 12 S_1 plants used in the earlier experiment. Considerable variation occurred in stem, leaf, fruit, and seed characters in the S_1 generation grown in the field.

Tetraploids of *Lycopersicon esculentum* varieties Danmark and Waltham Forcing produced relatively few seeds in fruits from flowers treated with pollen from lines 5 and 7 of diploid *L. peruvianum* P. I. 128645. Larger numbers of seeds were produced in fruits of these varieties, especially Waltham Forcing, from flowers pollinated with pollen from line 6. Similar results were obtained if pollen from these lines was used on flowers of tetraploid *L. pimpinellifolium*. Pollen from all three lines produced relatively large numbers of seeds in fruits set on the amphidiploid species hybrid.

There was little indication that these differences were caused by differences in meiotic instability in the different lines comparable with that reported by Lesley and Lesley (*11*). Although meiosis was not studied extensively in this material, chromosome counts demonstrated that all nine plants used here were normal diploids and no irregularities in meiosis were observed. Chromosome distribution at anaphases I and II was normal. Differences in average number of seeds in fruits

set by self-pollinated flowers did occur, but all nine plants produced abundant viable seeds.

The variation in efficiency of pollen in crosses with tetraploid *Lycopersicon esculentum* and *L. pimpinellifolium* among plants of diploid *L. peruvianum* P. I. 128645 and the variation in morphological characters in a field-grown progeny from a single plant both indicate considerable genetic diversity in this plant introduction. This is in agreement with the polymorphic nature of this species of *Lycopersicon* as described by Muller (*17*).

TETRAPLOID LYCOPERSICON ESCULENTUM × DIFFERENT COLLECTIONS OF DIPLOID L. PERUVIANUM

Taxonomic studies, plant-breeding experiments, and disease-reaction studies all indicate the diverse genetic nature of different collections of *Lycopersicon peruvianum*. It was therefore considered desirable to determine how different collections of it would respond in crosses with tetraploid *L. esculentum*. In the summer of 1946, three collections received as typical *L. peruvianum* P. I. 126926, P. I. 126944, and P. I. 128656 and two collections received as *L. peruvianum* var. *dentatum* P. I. 128645 and P. I. 128654 were used as pollen parents in crosses with tetraploid *L. esculentum* varieties Danmark and Waltham Forcing.[12]

There were considerable differences in the distribution of fruits among classes based on the size of the largest seed contained in each fruit (table 3). Many of the fruits set from flowers pollinated by

TABLE 3.—*Results from pollinations of tetraploid Lycopersicon esculentum by different collections of diploid L. peruvianum in the greenhouse at Cheyenne, Wyo., 1946*

Staminate parent	Flowers pollinated	Flowers setting fruits	Fruits with largest ovules or seeds in size class indicated					Total seeds		Average seeds per fruit, sizes 4 and 5
			Size 1	Size 2	Size 3	Size 4	Size 5	Size 4	Size 5	
	Number	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
P. I. 126926	37	86	1	7	15	7	2	26	2	0.88
P. I. 126944	39	72	1	2	5	7	13	16	13	1.04
P. I. 128645	23	70	3	0	3	1	9	36	43	4.94
P. I. 128654	55	91	2	14	30	3	1	7	2	.18
P. I. 128656	25	76	1	8	8	1	1	2	1	.16

Lycopersicon peruvianum P. I. 126944 and P. I. 128645 contained seeds in size classes 4 and 5. Seeds were less numerous in fruits from the former cross. Relatively fewer of the fruits set from flowers pollinated by P. I. 126926 contained seeds and most of these were placed in size class 4. Fruits set from flowers pollinated by P. I. 128656 and P. I. 128654 rarely contained seeds in size classes 4 and 5. Some

[12] P. I. 126434 and P. I. 126949, received as *Lycopersicon glandulosum*, were also used. The plants from both seed samples produced red fruits and were morphologically identical with *L. pimpinellifolium*. They reacted like diploid *L. pimpinellifolium* in crosses with diploid and tetraploid *L. esculentum* as reported by Cooper and Brink (*5*); that is, they were cross-fertile with diploid *L. esculentum* and nearly cross-sterile with its tetraploid.

extra large, plump seeds with the characteristic appearance of seeds containing sesquidiploid embryos were obtained from each cross.

These results indicate that sesquidiploid hybrids can be obtained from crosses between tetraploid *Lycopersicon esculentum* and various collections of diploid *L. peruvianum* and its varieties. However, the differences obtained from crosses involving different collections suggest that the degree of interfertility of a particular collection in such crosses cannot be predicted from present knowledge of this group.

DISCUSSION

Many workers have attempted to cross diploid *Lycopersicon esculentum* with diploid *L. peruvianum* and its varieties with varying degrees of success (p. 33). All collections of the 2 species that have been reported, including those reported herein, that have been studied cytologically have been found to have 12 pairs of chromosomes. The F_1 hybrids would therefore be expected to have 24 chromosomes (the diploid number). Lesley and Lesley (*11*) obtained diploid hybrids from crosses between diploid *L. esculentum* and Holmes' collection of *L. peruvianum* but obtained both triploid and amphidiploid plants in progenies derived from them. Dermen [13] found one of Porte and Walker's hybrids to be triploid.

The experiments reported herein demonstrate that sesquidiploid F_1 hybrids with 2 chromosome sets (24) from *Lycopersicon esculentum* and 1 chromosome set (12) from *L. peruvianum* can be obtained with relative ease. These results are in accord with those obtained by Cooper and Brink (*5*) from crosses between *L. pimpinellifolium* and *L. peruvianum* P. I. 128646 and P. I. 128649.

In practical breeding work it seems likely that the diploid and the sesquidiploid hybrids offer two modes of attack on the problem of incorporating into economically valuable tomatoes genetically controlled ability either to produce high ascorbic acid concentration or to exhibit disease resistance. The diploid hybrid may offer the possibility of exchange of chromosomes or parts of chromosomes between *Lycopersicon peruvianum* and *L. esculentum*. This is suggested by the cytological studies of Lesley and Lesley (*11*), who found apparently normal pairing at diakinesis and first metaphase in the diploid hybrids of *L. esculentum* × *L. peruvianum* and quadrivalent associations in amphidiploids. If pairing in the diploid hybrid is comparable with that observed in the parents, it should be possible to obtain new diploid forms from backcrosses to *L. esculentum* by using methods suggested by Smith (*23*).

The sesquidiploid offers the possibility of obtaining an entirely different type of tomato. Whether the 36 chromosomes form 12 trivalents, 12 bivalents and 12 univalents, or combinations between these extremes, most of the spores would have about 18 chromosomes with greater and smaller numbers occurring at lower frequencies. Lagging univalents would result in higher proportions of spores with the lower numbers of chromosomes. Occasional spores with 12 *Lycopersicon esculentum* chromosomes and 1 or 2 *L. peruvianum*

[13] Haig Dermen, associate cytologist, Division of Fruit and Vegetable Crops and Diseases, in personal discussion in 1943.

chromosomes should occur. From self-pollinations or from cross-pollinations with diploid *L. esculentum* these should yield aneuploids with 25 or 26 chromosomes ($2n + 1$ and $2n + 2$). The aneuploids in turn should yield on selfing 26-chromosome plants with 13 pairs of chromosomes. Although aneuploids could occur in progenies from diploid F_1 hybrids, they might be expected to occur more frequently in progenies from sesquidiploids. Of 12 possible aneuploid lines of this type some should be fertile and have potential value as cultigens with disease resistance or high vitamin content. If the aneuploids should prove to be sterile, they would still have value in determining the genetic factors involved in disease resistance. Such knowledge would be invaluable in the attack on the problem of utilizing disease resistance from this species in practical breeding programs.

A breeding program with the objective of obtaining aneuploid tomato lines has certain advantages and certain limitations that do not apply to a breeding program with the objective of obtaining diploid lines. With the latter objective it is possible that progress will be limited by sterility and testing difficulties in segregating populations. On the other hand, the possible number of new combinations may approach infinity. Regardless of how many offspring are obtained, tested, and found wanting in some essential character, there is always the possibility that a new individual with a new genotype will combine the desired potentialities.

With aneuploid lines as the objective, the breeding and testing programs may be simplified. Once obtained, a 26-chromosome (13-pair) aneuploid line should breed true, except for possible difficulties caused by the formation of trivalents or tetravalents and irregular chromosome distribution at meiosis. The aneuploid lines would permit the use of line tests rather than individual plant tests for fertility, disease resistance, high vitamin content, and other characters. When these characteristics have been determined for any one aneuploid line, the potential value of that line is thereby established. The potential number of 26-chromosome lines is, of course, limited.

Although natural aneuploid species or varieties are not known in the genus *Lycopersicon*, natural aneuploid series are known in other species or genera in many plant families including the Solanaceae. Gerstel (*6*) produced potentially valuable aneuploid lines in *Nicotiana*. The assumption that aneuploid tomato varieties can be produced from species hybrids is therefore supported by evidence from other genera.

SUMMARY

Interspecific cross-pollinations were made between diploid and tetraploid races of *Lycopersicon esculentum* and *L. peruvianum* P. I. 128645. Similar crosses were made by using *L. pimpinellifolium* or its F_1 hybrid with *L. esculentum* in place of *L. esculentum*. Tetraploid *L. esculentum* was also pollinated by diploid *L. peruvianum* P. I. 128654, P. I. 126926, P. I. 126944, and P. I. 128656. Each collection and tetraploid race was also self-pollinated.

Viable sesquidiploid seedlings were obtained from the cross tetraploid *Lycopersicon esculentum* pollinated by diploid *L. peruvianum* P. I. 128645. Similar results were obtained from tetraploid *L. pimpinellifolium* and its amphidiploid hybrid with *L. esculentum* pollinated by

the same collection of *L. peruvianum*. Seeds similar to those obtained from these crosses were obtained from tetraploid *L. esculentum* pollinated by *L. peruvianum* P. I. 126926, P. I. 126944, P. I. 128654, and P. I. 128656.

Other cross-pollinations produced no fruits or produced fruits with aborted ovules that failed to germinate from plantings of entire ovules or seeds in steamed soil or on an agar culture medium under aseptic conditions.

Marked seasonal variation in fruit-setting ability was found in diploid *Lycopersicon peruvianum* P. I. 128645, but not in its autotetraploid derivative or in diploid or tetraploid races of *L. esculentum* or *L. pimpinellifolium*. Variations were found also in morphological characters including number of seeds per fruit in inbred diploid lines of *L. peruvianum* P. I. 128645. These inbred lines also varied in seed-producing efficiency in crosses with tetraploid *L. esculentum*. Similar, but greater, differences in seed-producing efficiency in such crosses were found among different collections of *L. peruvianum*.

Fruit size of tetraploid *Lycopersicon peruvianum* exceeded that of the diploid. Fruit size of tetraploid *L. esculentum* exceeded that of the diploid only in fruits set by flowers pollinated by diploid *L. peruvianum*.

LITERATURE CITED

(1) Babb, M. F., and Kraus, J. E.
1939. Results of tomato variety tests in the Great Plains region. U. S. Dept. Agr. Cir. 533, 12 pp., illus.

(2) Bailey, D. M.
1941. The seedling test method for root-knot-nematode resistance. Amer. Soc. Hort. Sci. Proc. 38: 573–575.

(3) Blood, H. L.
1941. Solving Utah canning crop problems: the curly-top breeding program. Utah Agr. Col. Ext. [Cir.] (n. s.) 109, pp. 26–29.

(4) Bohn, G. W.
1947. Colchicine treatments for use with tomatoes. Jour. Hered. 38: 157–160, illus.

(5) Cooper, D. C., and Brink, R. A.
1945. Seed collapse following matings between diploid and tetraploid races of Lycopersicon pimpinellifolium. Genetics 30: 376–401, illus.

(6) Gerstel, D. U.
1945. Inheritance in Nicotiana tabacum. XX. The addition of Nicotiana glutinosa chromosomes to tobacco. Jour. Hered. 36: 197–206, illus.

(7) Goodspeed, T. H., and Bradley, M. V.
1942. Amphidiploidy. Bot. Rev. 8: [271]–316.

(8) Holmes, F. O.
1939. The Chilean tomato, Lycopersicon chilense, as a possible source of disease resistance. (Phytopath. note) Phytopathology 29: 215–216.

(9) ———
1943. A tendency to escape tobacco-mosaic disease in derivatives from a hybrid tomato. Phytopathology 33: 691–697, illus.

(10) Lesley, J. W.
1935. A tomato relative from Peru. Jour. Hered. 26: 451–453, illus.

(11) Lesley, M. M., and Lesley, J. W.
1943. Hybrids of the Chilean tomato: sterile and fertile plants of Lycopersicon peruvianum var. dentatum Dun. (L. chilense Dun.) and diploid and tetraploid hybrids with cultivated tomatoes. Jour. Hered. 34: 199–205, illus.

(12) Lincoln, R. E., Zscheile, F. P., Porter, J. W., and others.
1943. provitamin a and vitamin c in the genus lycopersicon. Bot. Gaz. 105: 113–115.
(13) Lindstrom, E. W., and Humphrey, L. M.
1933. comparative cyto-genetic studies of tetraploid tomatoes from different origins. Genetics 18: 193–209, illus.
(14) ——— and Koos, K.
1931. cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot. 18: 398–410, illus.
(15) MacArthur, J. W., and Chiasson, L. P.
1947. cytogenetic notes on tomato species and hybrids. Genetics 32: 165–177, illus.
(16) McFarlane, J. S., Hartzler, E., and Frazier, W. A.
1946. breeding tomatoes for nematode resistance and for high vitamin c content in hawaii. Amer. Soc. Hort. Sci. Proc. 47: 262–270, illus.
(17) Muller, C. H.
1940. a revision of the genus lycopersicon. U. S. Dept. Agr. Misc. Pub. 382, 29 pp., illus.
(18) Oba, G. I., Riner, M. E., and Scott, D. H.
1945. experimental production of hybrid tomato seed. Amer. Soc. Hort. Sci. Proc. 46: 269–276, illus.
(19) Oka, T. H.
1936. the progeny of tetraploid tomatoes. Jap. Jour. Genet. 12: 89–92, illus. [In Chinese. English summary, pp. 91–92.]
(20) Porte, W. S., and Walker, H. B.
1945. a cross between lycopersicon esculentum and disease-resistant l. peruvianum. (Phytopath. note) Phytopathology 35: 931–933, illus.
(21) Reynard, G. B., and Kanapaux, M. S.
1942. ascorbic acid (vitamin c) content of some tomato varieties and species. Amer. Soc. Hort. Sci. Proc. 41: 298–300.
(22) Smith, P. G.
1944. embryo culture of a tomato species hybrid. Amer. Soc. Hort. Sci. Proc. 44: 413–416, illus.
(23) ———
1944. reaction of lycopersicon spp. to spotted wilt. (Phytopath. note) Phytopathology 34: 504–505.
(24) Upcott, M.
1935. the cytology of triploid and tetraploid lycopersicum esculentum. Jour. Genet. 31: 1–19, illus.
(25) Virgin, W. J.
1940. the chilean tomato, lycopersicon chilense, found resistant to curly top. (Phytopath. note) Phytopathology 30: 280.
(26) ———
1942. promising tomato hybrid tested. Idaho Agr. Expt. Sta. Ann. Rpt. (1941) 49 (Bul. 244): 41.
(27) Winkler, H.
1916. über die experimentelle erzeugung von pflanzen mit abweichenden chromosomenzahlen. Ztschr. f. Bot. 8: 417–544, illus.
(28) Wright, V., and Lincoln, R. E.
1940. resistance to defoliation diseases in tomato. Ind. Agr. Expt. Sta. Ann. Rpt. 53: 42–43, illus.
(29) Yeager, A. F., and Hepler, J. R.
1941. plant breeding studies. N. H. Agr. Expt. Sta. Ann. Rpt. 1940 (Bul. 330): 35–36.
(30) ——— and Purinton, H. J.
1946. lycopersicon peruvianum as a parent in the development of high ascorbic acid tomato varieties. Amer. Soc. Hort. Sci. Proc. 48: 403–405, illus.

SEASONAL VARIATION OF CAROTENE AND OTHER NUTRITIONALLY IMPORTANT CONSTITUENTS IN THE TWO PASTURE GRASSES DALLIS AND CARPET [1]

By J. R. KUPPERS, formerly *assistant in dairy nutrition*, L. L. RUSOFF, *associate dairy nutritionist*, and D. M. SEATH, *head, Dairy Research Department, Louisiana Agricultural Experiment Station* [2]

INTRODUCTION

Carpet grass (*Axonopus affinis* Chase) and Dallis grass (*Paspalum dilatatum* Poir.) are species of considerable economic importance in the Gulf coastal plain region. Since information concerning their composition is meager, analyses were made to determine the trends of carotene, protein, lignin, cellulose, calcium, phosphorus, ash, and moisture in the two grasses. The samples were collected from a pasture which was fertilized, clipped for weed control, and was being closely grazed according to a rotational system over a growing season of 9 months. A comparison was made of the behavior of the two species with respect to the constituents determined, and the data were examined for interrelationships between the trends of the different constituents, with special reference to carotene.

REVIEW OF LITERATURE

The factors which have been said to influence the carotene content of plants are: Stage of maturity (*28*),[3] drought (*16*, *18*), intensity of light and temperature (*2*), and intensity of grazing (*13*, *23*). In general, the work of others bears out the statement made by Virtanen (*27*) in 1936 that the carotene content of plants is closely related to growth, that optimum growth occurs simultaneously with maximum carotene content, and that factors which retard growth affect the carotene content adversely.

The crude protein content of plants has also been found to decline consistently with advancing stages of maturity, and highly significant correlations have been obtained between the crude carotene and crude protein content of several grasses and grains (*9*, *14*, *15*, *22*, *23*, *26*).

[1] Received for publication November 5, 1947.

[2] The analyses for crude protein reported herein were performed by Frances L. Bonner, and the ether extraction of the grass samples, prior to the determination of lignin, was made by Clayton C. Moreland, both of the Feeds and Fertilizer Laboratory of this station. Stephen Lichtblau, official in charge, Weather Bureau, U. S. Department of Commerce, New Orleans, La., furnished much of the meteorological data presented in this paper.

[3] Italic numbers in parentheses refer to Literature Cited, p. 62.

Determinations of the content of lignin and cellulose by different methods have indicated that both constituents increase steadily with advancing stages of growth (*19*, *20*, *24*).

Reports that the phosphorus content of plants decreases with the more advanced stages of maturity are reasonably consistent. The calcium content of grasses, on the other hand, has been found by some workers to increase (*8*, *12*), by others to decrease (*4*, *7*, *21*), and by still others to be quite variable (*13*, *24*) as the plant approaches maturity. Investigations of the effect of rainfall on the calcium and phosphorus content of grasses are generally in agreement with the statement that the phosphorus content is directly proportional while the calcium content is inversely proportional to rainfall. Hart and Guilbert (*7*), however, have reported that leaching decreased the content of both minerals.

PROCEDURE

The experimental pasture from which the grass samples were collected is located on a farm at Mount Hermon in Washington Parish, La. The predominant soil type is Cahaba fine sandy loam. Since 1941 the pasture had received phosphorus and potash fertilizers semiannually, with dolomitic limestone (30.3 percent CaO, 21 percent MgO) applied in the fall of 1941 and 1944 at the rate of 2 tons per acre. During the fall of 1945 preceding this experiment applications were made of calcium metaphosphate (60 percent P_2O_5, 25.8 percent CaO) at the rate of 200 pounds per acre and muriate of potash (50 percent K_2O) at 100 pounds per acre. The pasture was clipped periodically to control weeds. It supported principally white Dutch and hop clovers, and carpet, Dallis, and Bermuda grasses.

During the growing season, March to November 1946, samples of carpet and Dallis grass were gathered on 10 different days. Between the first and second collections there was an interval of 2 weeks, and between the second and third and the third and fourth collections there was an interval of 3 weeks. Thereafter the interval was 4 weeks.

Twenty to one hundred grams of fresh material from each species of grass were gathered from four plots of 0.8 acre each, two of which had been plowed every 2 years and two of which had not been cultivated. The plots were being closely grazed to a height of about 3 inches, in rotation, by dairy cattle. Each plot was systematically sampled in a random manner by crisscrossing. Whole stems of the plants were clipped individually with a jackknife approximately 1 inch above the base of the plant. The samples were intended to represent the green forage available for grazing at the time of sampling. No dead material was gathered and areas surrounding dung heaps were avoided. At times cattle were grazing on a plot when the samples were taken.

The fresh material, 1 to 5 inches in length, was cut into ½-inch lengths with shears, and a 5-gm. sample was used for the carotene determination. The other analyses were made on the air-dry material which had been ground to pass a 1-mm. mesh sieve.

Carotene was determined by the method of Moore and Ely (*16*), crude protein by multiplying the total Kjeldahl nitrogen by 6.25 (*1*), lignin and cellulose by the method of Crampton and Maynard (*3*), and calcium and phosphorus by the method of Morris, Nelson, and

Palmer (*17*). A 500° C. ash was obtained (*1*). The loss of moisture on air drying at room temperature and the loss on heating at 100° C. for 5 hours under a pressure not exceeding 100 mm. of mercury (*1*) were recorded.

Precipitation was recorded on the experimental pasture, and temperature data were obtained from the United States Weather Bureau station at Tylertown, Miss., about 15 miles from the pasture.

Coefficients of correlation between the trends of some plant constituents were computed.

RESULTS

Table 1 gives the range and averages by collection dates of the content of crude carotene, crude protein, lignin, cellulose, calcium, phosphorus, and ash in Dallis and carpet grasses collected during the growing season of 1946 from the experimental pasture.

The average carotene content of carpet grass (table 1) did not vary greatly throughout the season, except for a decided rise to a maximum value of 257 p. p. m. in July. A minimum of 94 p. p. m. occurred in April. The over-all trend of carotene in Dallis grass was downward from a maximum average of 464 p. p. m. in April to a minimum of 113 p. p. m. in September. Two prominent breaks in this trend were noted; one came in July, when the value rose to a secondary maximum of 403 p. p. m., and the other in October with a tertiary maximum of 312 p. p. m.

Protein values of carpet grass showed no detectable trend. A maximum average of 12.8 percent was found in June and a minimum of 8.5 percent in April. For Dallis grass the average protein content followed a regular downward trend from a maximum of 18.6 percent in March to a minimum of 9.1 percent in September. There was a subsequent rise in October to 14.9 percent.

Lignin showed a steady increase in both species with minimum average values of 10.1 percent for carpet grass and 12.5 percent for Dallis grass in the spring and with maximum values of 15.3 percent for carpet and 19.9 percent for Dallis in the fall.

Cellulose values for both species followed a trend similar to that for lignin, with the exception of a marked decline in October from the September values. Minimum average values were 31.1 percent for carpet and 29.6 percent for Dallis; maximum average values were 38.1 percent for carpet and 42.3 percent for Dallis.

The calcium content of carpet grass showed no definite trend. The maximum and minimum average values were 0.44 and 0.31 percent, respectively. In Dallis grass calcium decreased steadily from a maximum of 0.55 percent in March to a minimum of 0.29 percent in September, followed by a marked increase in October to 0.41 percent.

Phosphorus values for both species were low in April, rising to a maximum average of 0.49 percent for carpet and 0.69 percent for Dallis in May, then declining steadily to a minimum of 0.34 percent for carpet and 0.31 percent for Dallis in September. A subsequent rise was found in October to a value of 0.39 percent for both species.

Ash values for both species followed trends similar to those for phosphorus. Maximum average values were 8.75 percent for carpet and 10.86 percent for Dallis. Minimum average values were 5.82 percent for carpet and 6.79 percent for Dallis.

TABLE 1.—*Range and averages by collection dates of crude carotene, crude protein, lignin, cellulose, calcium, phosphorus, and ash on the dry basis, and moisture in carpet and Dallis grasses collected during the growing season of 1946 from an improved pasture in Louisiana*

DALLIS GRASS [1]

Collection date	Carotene		Protein		Lignin		Cellulose		Calcium		Phosphorus		Ash		Moisture	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	P. p. m.	*P. p. m.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Mar. 29	389	293–503	18.6	18.1–19.1	12.5	8.7–14.7	29.6	27.3–31.2	0.55	0.50–0.61	0.44	0.41–0.47	7.18	6.82– 7.81	71.1	69.4–75.4
Apr. 8	464	370–515	17.6	16.0–19.1	13.7	12.1–14.4	33.8	33.0–34.5	.49	.44– .55	.44	.36– .52	9.67	8.70–10.47	74.0	71.3–76.1
Apr. 29	281	189–379	14.0	10.1–16.3	15.9	14.8–16.7	37.0	35.6–38.7	.42	.37– .49	.40	.35– .45	8.65	8.21– 8.86	73.2	69.5–75.6
May 20	301	214–446	15.9	12.6–19.5	17.4	16.4–18.5	36.5	33.0–39.0	.38	.32– .44	.69	.53– .78	10.86	10.16–12.30	78.9	78.5–79.2
June 17	180	120–229	11.3	7.5–14.3	17.2	16.8–17.9	39.3	37.5–41.7	.35	.33– .36	.44	.37– .49	8.61	8.21– 9.32	74.1	71.3–77.3
July 15	403	339–468	12.8	10.6–13.6	17.6	17.1–18.9	41.4	40.5–42.9	.33	.32– .35	.38	.35– .40	7.62	6.61– 8.16	77.4	76.3–78.2
Aug. 12	116	108–129	11.3	10.7–12.1	19.3	18.2–20.0	41.1	40.3–41.4	.34	.31– .38	.34	.32– .36	7.18	6.92– 7.31	66.3	57.1–72.5
Sept. 9	113	81–145	9.1	8.3– 9.5	18.3	17.4–19.5	42.3	41.4–43.0	.29	.27– .30	.31	.27– .34	6.79	6.02– 7.43	68.1	67.0–69.7
Oct. 8	312	293–333	14.9	12.9–16.9	19.9	19.1–20.5	36.4	35.5–37.4	.41	.38– .45	.39	.38– .41	8.81	8.50– 9.45	68.5	65.5–70.6
Nov. 7	285	262–312	14.1	13.0–15.0	18.7	18.2–19.1	38.8	38.4–39.7	.41	.40– .42	.37	.33– .40	9.16	8.61– 9.66	73.4	70.6–74.6
CARPET GRASS [2]																
Apr. 8	137	117–156	9.5	9.4– 9.7	10.1	9.7–10.4	31.5	31.4–31.5	0.44	0.43–0.46	0.33	0.30–0.36	5.82	5.60– 6.00	67.1	64.9–69.3
Apr. 29	94	72–116	8.5	7.5–10.4	10.6	10.4–10.9	31.1	29.8–32.5	.33	.23– .39	.36	.33– .38	6.87	6.82– 7.92	60.8	56.2–62.2
May 20	133	85–183	10.6	8.2–13.1	11.4	10.3–12.3	33.1	30.7–34.8	.37	.35– .41	.49	.45– .53	8.75	7.61–10.23	67.6	55.0–71.4
June 17	151	97–244	12.8	10.3–16.1	11.0	10.4–12.0	33.7	32.7–34.6	.36	.29– .42	.42	.39– .44	8.24	7.91– 8.48	67.3	63.1–71.6
July 15	257	173–338	10.8	9.5–11.9	11.9	10.3–13.0	35.3	33.9–37.0	.41	.37– .45	.38	.37– .40	6.67	5.87– 7.21	72.7	69.4–77.2
Aug. 12	126	96–151	11.8	10.6–13.1	13.1	12.3–14.1	35.7	34.8–37.5	.44	.42– .46	.36	.32– .40	7.10	6.22– 8.05	64.8	59.2–70.3
Sept. 9	112	78–163	10.1	9.3–10.9	14.9	13.9–15.5	38.1	37.0–38.7	.38	.35– .39	.34	.32– .37	6.79	6.24– 7.21	68.0	66.1–71.2
Oct. 8	152	121–200	10.5	9.7–12.1	15.1	14.5–16.2	36.3	35.4–37.1	.31	.29– .32	.39	.37– .45	7.13	6.53– 8.07	70.2	67.9–72.5
Nov. 7	137	118–156	11.1	10.9–11.2	15.3	14.3–16.7	36.4	35.8–36.8	.35	.34– .38	.38	.37– .39	7.64	7.01– 7.99	74.2	72.5–75.4

[1] Total number of analyses: 40 for all constituents.
[2] Total number of analyses: 31 for carotene; 33 for all other constituents.

The moisture content of carpet and Dallis grasses was high in the late fall and after periods of heavy rainfall. The seasonal average values were 68.1 percent for carpet and 72.5 percent for Dallis grass. Meteorological data for the period of the investigation are shown in table 2.

TABLE 2.—*Meteorological data for the growing season of 1946*

Period	Daily temperatures [1]		Rainfall [2]	Mean length of day [3]
	Mean maximum	Mean minimum		
	°F.	°F.	*Inches*	*Hours and minutes*
Mar. 29–Apr. 7	83	58	0	12:32
Apr. 8–Apr. 28	79	57	.75	13:02
Apr. 29–May 19	80	61	8.37	13:36
May 20–June 16	85	64	2.38	14:02
June 17–July 14	88	70	13.25	14:01
July 15–Aug. 11	89	71	9.06	13:42
Aug. 12–Sept. 8	89	67	1.00	12:59
Sept. 9–Oct. 7	83	64	4.25	12:07
Oct. 8–Nov. 6	81	57	1.00	11:33

[1] Temperatures as recorded by the U. S. Weather Bureau at Tylertown, Miss., about 15 miles from the experimental pasture.
[2] Rainfall data as recorded on the experimental pasture at Mount Hermon, La.
[3] Length of day (sunrise to sunset) taken from data compiled by the U. S. Naval Observatory.

TABLE 3.—*Coefficients of correlation (r) interrelating the content of carotene, protein, lignin, cellulose, and calcium in carpet and Dallis grasses collected during the growing season of 1946 from an improved pasture in Louisiana under actual grazing conditions*

Species	Number of comparisons	Protein	Lignin	Cellulose
		r	r	r
Dallis	40—Carotene	+0.727**	−0.525**	−0.619**
	40—Protein		−.561**	−.790**
	40—Lignin			+.714**
	40—Calcium	+.788**		
Carpet	31—Carotene	+.521*	−.155	−.058
	33—Protein		−.024	−.008
	33—Lignin			+.253
	33—Calcium	+.123		

*Indicates $P<0.01$; **indicates $P<0.05$.

Coefficients of correlation interrelating the content of carotene, protein, lignin, cellulose, and calcium are presented in table 3. Highly significant correlations were found between crude carotene and crude protein, and lignin and cellulose for Dallis grass, while only a significant correlation was found between crude carotene and crude protein for carpet grass.

DISCUSSION

Two common methods of gathering plant material from pastures or range lands for seasonal studies are (1) collecting the herbage according to stage of growth and (2) mowing a selected plot at set intervals. The method used in this investigation was similar to the latter except that the material was collected from a plot which was being heavily grazed by dairy cattle, and the grasses were collected from the same plot in an uncontaminated condition, 100 percent pure,

with respect to species. Material collected by this method necessarily contains a mixture of several stages of growth. The quantity of the more advanced stages of growth collected at any one time depends upon the rate at which growth matures and the rate at which new growth is stimulated from one collection date to the next. There were no extremely mature plants in the collections since the pasture was mowed three or four times during the season to control weeds.

It has been reported by many workers (*13*, *14*, and others) that the carotene content of grasses is highest in young, rapidly growing plants. Thus, in studying an actively growing pasture in which vegetation eaten by the grazing animal is continually being replenished by new growth, it is to be expected that carotene content should be highest during the periods that are most favorable for rapid growth of the plants. If we choose that portion of the 1946 season most favorable for rapid growth according to the carotene content, it is seen that the early spring was best suited for Dallis grass, while midsummer was best suited for carpet grass (table 1).

In the present investigation the response of carotene content to rainfall was unpredictable. Decided rises in carotene content with increasing rainfall occurred only during the hot months when a reduction of illumination might have proved favorable for pigment production. Also, an increased carotene content occurred with the shorter and cooler days of fall. Thus, the data presented herein are not inconsistent with the suggestion of Beck and Redman (*2*) that there is an optimum temperature and intensity of illumination for the production of plant pigments, and that soil moisture plays a minor role.

Carotene values reported by Kemmerer et al. (*10*) for three samples of fresh Dallis grass fall within the range of values reported for this study.

Attention should be called to the wide variation in carotene values (table 1) of samples from four different plots collected on the same day. The differences between plots with respect to the carotene content of the grasses were not consistent, and any isolated determination is therefore of little significance. However, a seasonal trend may be discerned from the accumulation of these values over the growing season.

There is a similarity between the seasonal trends of carotene and protein within each species (table 1), but between species the trends are quite different. The relationships between the content of carotene and protein of the two grasses are indicated by the coefficients of correlation, +0.73 for 40 samples of Dallis grass and +0.52 for 31 samples of carpet grass. These correlations are lower than those reported for various herbages cut according to stage of growth (*9*, *14*, *15*, *22*, *23*), but they compare favorably with the correlation of +0.53 reported by Thomas and Moon (*26*) for 46 samples of mixed herbage collected by periodic mowing of plots at set intervals.

For Dallis grass, which has the greater seasonal variation in composition, the coefficients of correlation interrelating the carotene, protein, lignin, cellulose, and calcium are all highly significant. However, in the case of carpet grass, which has less seasonal variation, the only significant correlation was between protein and carotene. This is presented as strengthening evidence for the parallelism between the

production of carotene and an increasing content of total nitrogen in plant material.

Lush (*11*) stated that the seasonal trends of protein in mixed grasses and clovers from Louisiana pastures were from high spring values to low midsummer values. In the present study this was true of Dallis grass but not of carpet grass (table 1).

Stansel et al. (*25*), in pasture studies on the Gulf Coast prairie of Texas, collected samples from pure stands of carpet and Dallis grass cut at monthly intervals with a lawn mower. Although the mowed grass was higher in protein content than grass from unmowed areas they observed no seasonal trend in the protein content of either species. Their protein values for 15 collections over two growing seasons averaged 9.2 percent for carpet grass and 10.3 percent for Dallis grass. The grand averages found in the present investigation are considerably greater for Dallis grass. These values are 10.6 percent for carpet grass and 14.0 percent for Dallis grass.

Seasonal trends of lignin and cellulose obtained in this study were similar for both species. Relatively high values for lignin were reached early in the season. The over-all average for the lignin content of carpet grass was considerably lower than that for Dallis grass, indicating a lower content of undigestible carbohydrates in that species. It is suggested that lignin and cellulose values may be used as an approximate index to the average stage of maturity of the grass in any one sample collected during this investigation.

The seasonal trends in calcium content of carpet and Dallis grass are quite dissimilar (table 1). Calcium values presented in this paper fall within the range reported by Texas workers (*5*, *6*, *25*) for carpet and Dallis grass, i. e., 0.23–0.46 percent for carpet grass and 0.25–0.61 percent for Dallis grass.

Maximum phosphorus values of carpet and Dallis grass were reached in May following a period during which there was 8.37 inches of rain, but other periods of unusually heavy rainfall were not followed by a similar response. The two species appear to respond similarly to varying climatic and environmental conditions with respect to phosphorus content.

Phosphorus values from this investigation are considerably higher than those reported by the Texas investigators (*5*, *6*, *25*), which were 0.03–0.14 percent for carpet grass and 0.04–0.16 for Dallis grass. The differences may have been due principally to good pasture management, and especially to the application of phosphate fertilizers to the experimental plots, for the Texas experiments were carried out on phosphorus-deficient soil.

SUMMARY

A study was made of the seasonal trends of some nutritionally important constituents of two species of pasture grass, carpet (*Axonopus affinis*) and Dallis (*Paspalum dilatatum*), during a growing season of 9 months. The pasture plots had been fertilized, clipped at regular intervals to prevent weed growth, and heavily grazed in rotation by dairy cattle.

On an average and over the greater part of the season, Dallis grass had a higher content of crude carotene, crude protein, lignin, cellulose,

calcium, phosphorus, ash, and moisture. However, during the hot months of August and September carpet grass was as good as or better than Dallis grass as a source of carotene, protein, calcium, and phosphorus. Seasonal maxima in the content of carotene, protein, and calcium occurred in early spring for Dallis grass, and in early summer for carpet grass.

Seasonal variation in composition was less for carpet grass than for Dallis grass. Carotene, protein, phosphorus, and ash values for both species were comparatively low in late summer and rose with the advent of cooler fall weather. The moisture content of the two species increased consistently with increasing rainfall.

Highly significant correlations were found between crude carotene and crude protein in both carpet grass (+0.52) and Dallis grass (+0.73). Other correlations measuring relationship between organic constituents were highly significant for Dallis grass but not for carpet grass. There was a difference between species in the general trends of carotene, protein, and calcium, but no consistent differences were apparent in the trends of lignin, cellulose, phosphorus, ash, and moisture.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1940. Official and tentative methods of analysis . . . Ed. 5, 757 pp., illus. Washington, D. C.

(2) Beck, W. A., and Redman, R.
1940. Seasonal variation in the production of plant pigments. Plant Physiol. 15: 81–94, illus.

(3) Crampton, E. W., and Maynard, L. A.
1938. The relation of cellulose and lignin content to the nutritive value of animal feeds Jour. Nutr. 15: 383–395.

(4) Daniel, H. A., and Harper, H. J.
1935. The relation between effective rainfall and total calcium and phosphorus in alfalfa and prairie hay. Amer. Soc. Agron. Jour. 27: 644–652, illus.

(5) Fraps, G. S., and Fudge, J. F.
1940. The chemical composition of forage grasses of the east Texas timber country. Tex. Agr. Expt. Sta. Bul. 582, 35 pp.

(6) Fudge, J. F., and Fraps, G. S.
1944. The chemical composition of forage grasses from the Gulf Coast prairie as related to soils and to requirements for range cattle. Tex. Agr. Expt. Sta. Bul. 644, 39 pp.

(7) Hart, G. H., and Guilbert, H. R.
1928. Factors influencing percentage calf crop in range herds. Calif. Agr. Expt. Sta. Bul. 458, 43 pp., illus.

(8) ———Guilbert, H. R., and Goss, H.
1932. Seasonal changes in the chemical composition of range forage and their relation to nutrition of animals. Calif. Agr. Expt. Sta. Bul. 543, 62 pp., illus.

(9) Keirstead, L. G.
1945. Relation of carotene and crude protein content of grasses. (Note) Amer. Soc. Agron. Jour. 37: 239–240, illus.

(10) Kemmerer, A. R., Fudge, J. F., and Fraps, G. S.
1942. Carotene content of Texas range forage. Amer. Soc. Agron. Jour. 34: 851–855.

(11) Lush, R. H.
1938. Seasonal composition and yields of pastures. La. Agr. Expt. Sta. Bul. 299, 24 pp.

(12) McCreary, O. C.
1931. Wyoming forage plants and their chemical composition. Wyo. Agr. Expt. Sta. Bul. 184, 23 pp., illus.

(13) McMillen, W. N., Williams, Q., and Langham, W.
1943. CHEMICAL COMPOSITION, GRAZING VALUE AND VEGETATIVE CHANGES OF HERBAGE IN A TYPICAL PLAINS PASTURES. Jour. Anim. Sci. 2: [237]–243.

(14) Moon, F. E.
1939. THE COMPOSITION OF GRASS AT VARIOUS STAGES OF MATURITY, AND THE CHANGES OCCURRING DURING HAYMAKING, WITH PARTICULAR REFERENCE TO CAROTENE-CONTENT. Empire Jour. Expt. Agr. 7: [225]–234, illus.

(15) ———
1939. THE INFLUENCE OF MANURIAL TREATMENT ON THE CAROTENE CONTENT POOR PASTURE GRASS, AND ON RELATIONSHIP OF THIS CONSTITUENT TO THE ASH AND ORGANIC FRACTIONS. Jour. Agr. Sci. [England] 29: [524]–543.

(16) Moore, L. A., and Ely, R.
1941. EXTRACTION OF CAROTENE FROM PLANT MATERIAL . . . Indus. and Engin. Chem., Analyt. Ed. 13: 600–601, illus.

(17) Morris, H. P., Nelson, J. W., and Palmer, L. S.
1931. QUANTITATIVE DETERMINATION OF CALCIUM, MAGNESIUM, AND PHOSPHORUS IN FEEDSTUFFS AND CATTLE EXCRETA . . . Indus. and Engin. Chem., Analyt. Ed. 3: 164–167. 1931.

(18) Myburgh, S. J.
1935. I. THE CAROTENE CONTENT OF SOME SOUTH AFRICAN FEEDS. Onderstepoort Jour. Vet. Sci. and Anim. Indus. 5: 475–482.

(19) Patton, A. R.
1943. SEASONAL CHANGES IN THE LIGNIN AND CELLULOSE CONTENT OF SOME MONTANA GRASSES. II. Jour. Anim. Sci. 2: [59]–62.

(20) ——— and Gieseker, L.
1942. SEASONAL CHANGES IN THE LIGNIN AND CELLULOSE CONTENT OF SOME MONTANA GRASSES. Jour. Anim. Sci. 1: [22]–26.

(21) Richardson, A. E. V., Trumble, H. C., and Shapter, R. E.
1931. FACTORS AFFECTING THE MINERAL CONTENT OF PASTURES . . . Austral. Council Sci. and Indus. Res. Bul. 49, 47 pp., illus.

(22) Smith, A. M., and Robb, W.
1943. THE CAROTENE AND PROTEIN IN OATS AND BARLEY AT DIFFERENT STAGES OF GROWTH. Jour. Agr. Sci. [England] 33: 119–121.

(23) ——— and Wang, T.
1941. THE CAROTENE CONTENT OF CERTAIN SPECIES OF GRASSLAND HERBAGE. Jour. Agr. Sci. [England] 31: [370]–378, illus.

(24) Stanley, E. B., and Hodgson, C. W.
1938. SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF SOME IMPORTANT ARIZONA RANGE GRASSES. Ariz. Agr. Expt. Sta. Tech. Bul. 73, pp. 451–466, illus.

(25) Stansel, R. H., Reynolds, E. B., and Jones, J. H.
1939. PASTURE IMPROVEMENT IN THE GULF COAST PRAIRIE OF TEXAS. Texas Agr. Expt. Sta. Bul. 570, 43 pp.

(26) Thomas, B., and Moon, F. E.
1938. A PRELIMINARY STUDY OF THE EFFECTS OF MANURIAL TREATMENT, AND OF AGE, ON THE CAROTENE-CONTENT OF GRASS. Empire Jour. Expt. Agr. 6: [235]–244.

(27) Virtanen, A. I.
1936. VITAMINS AND PLANTS. Nature [London] 137: 779–780.

(28) ——— Hausen, S. v., and Saastamoinen, S.
1933. UNTERSUCHUNGEN ÜBER DIE VITAMINBILDUNG IN PFLANZEN. I. Biochem. Ztschr. 267: [179]–191, illus. [Abstract in Chem. Abs. 28: 1740. 1934.]

U. S. GOVERNMENT PRINTING OFFICE: 1948